Docket No.: 209013US

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

JOHN B LOWE : GROUP ART UNIT: 1652

SERIAL NO: 09/863,475 : EXAMINER: PROUTY

FILED: MAY 24, 2001 :

FOR: METHODS AND PRODUCTS FOR THE SYNTHESIS OF OLIGOSACCHARIDE STRUCTURES ON GLYCOPROTEINS, GLYCOLIPIDS, OR AS FREE MOLECULES, AND FOR THE ISOLATION OF CLONED GENETIC SEQUENCES THAT DETERMINE THESE STRUCTURES

DECLARATION

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Now comes John B. Lowe, who deposes and states that:

1. I am the sole inventor of the subject matter described and claimed in U.S. Patent Application Serial Number 09/863,475 (hereinafter referred to as "the '475 application").

2. I am a co-author of the publication Larsen et al., *Proceedings of the National Academy of Sciences*, Vol. 86, 8227-8231, November 1989 (hereafter referred to as "the Larsen et al. publication").

3. I am the sole inventor of the subject matter common to both the '475 application and the Larsen et al. publication.

4. The other authors of the Larsen et publication, Robert D. Larsen, Valanila P. Rajan, Melissa M. Ruff, Jolanta Kukowska-Latallo, and Richard D. Cummings, made no inventive contribution to the subject matter claimed in the '475 application, and, therefore, are not co-inventors in that application.

5. Below, I provide a description of the contributions that each author of the Larsen et al. publication made to the work described in the '475 application and the Larsen et al. publication.

A. John B. Lowe

(i) I was the laboratory director in the Howard Hughes Medical Institute at the University of Michigan.

(ii) I conceived of the general method to use COS cells to isolate functional cDNAs encoding an alpha 1,3-galactosyltransferase cDNA.

(iii) I made the decision as to which expression vector would be used to construct the cDNA libraries that were screened.

(iv) I directed a technician, Ms. Melissa Ruff in experiments to optimally transform the *E. coli* host used to construct the cDNA libraries, and used to rescue the vector from the host cells using the Hirt supernatant procedure.

(v) I directed Ms. Ruff in the actual screening of the libraries, including examining "panned" transfected cells after each round of transfection, and identifying by this procedure which pool of cDNA clones should be further amplified and transfected as smaller pools.

(vi) I designed the sibling selection procedure used to isolate the cDNA clones that contained the alpha 1,3-galactosyltransferase cDNA, and directed Ms. Ruff in this activity.

(vii) I directed Ms. Ruff in her efforts to determine the DNA sequence of the alpha 1,3-galactosyltransferase cDNA.

(viii) I read virtually all of the DNA sequencing gels generated by Ms. Ruff, and analyzed the DNA sequence derived from these gels, and those read by her, including generation of hydropathy plots.

(ix) I designed the protein A fusion vector strategy used to create a protein A-alpha-1,3-galactosyltransferase expression vector, and prepared the early stages of this construct.

(x) I directed Dr. Robert Larsen, a post-doctoral fellow in my laboratory, in constructing the protein A fusion vector.

(xi) I directed Dr. Larsen and Dr. Rajan in assaying extracts and conditioned media taken from cells transfected with the protein A-alpha 1,3-galactosyltransferase expression vector.

(xii) I directed Dr. Larsen and Dr. Rajan in preparing 14C-labeled product of the protein A-alpha 1,3-galactosyltransferase.

(xiii) I requested Dr. Cummings and his colleagues to confirm the structure of the 14C-labeled product of the protein A-alpha 1,3-galactosyltransferase.

(xiv) I wrote the entire manuscript that was published as the Larsen et al. publication, and prepared the figures in that manuscript.

B. Robert D. Larsen

(i) Dr. Larsen was a post-doctoral fellow in my laboratory in the Howard Hughes Medical Institute at the University of Michigan.

(ii) Dr. Larsen prepared the cDNA expression library used to isolate the alpha 1,3-galactosyltransferase cDNA, using a donor cell chosen by myself and Dr. Cummings.

(iii) Under my direction, Dr. Larsen completed construction of the protein A-alpha 1,3-galactosyltransferase expression vector.

(iv) Under my direction, Dr. Larsen contributed to expressing the alpha 1,3-galactosyltransferase cDNA and the protein A-alpha 1,3-galactosyltransferase expression vectors in COS cells.

(v) Under my direction, Dr. Larsen contributed to FACS analysis of COS cells expressing the alpha 1,3-galactosyltransferase cDNA.

(vi) Dr. Larsen completed the Northern blot (data used in Figure 5 of the Larsen et al. publication).

C. Valanila P. Rajan

(i) Dr. Rajan was a senior research technician in my laboratory in the Howard Hughes Medical Institute at the University of Michigan.

(ii) Under my direction, Dr. Rajan contributed to expressing the alpha 1,3-galactosyltransferase cDNA and the protein A-alpha 1,3-galactosyltransferase expression vectors in COS cells.

(iii) Dr. Rajan assayed extracts and conditioned media taken from cells transfected with the protein A-alpha 1,3-galactosyltransferase expression vector.

(iv) Dr. Rajan prepared 14C-labeled product of the protein A-alpha 1,3-galactosyltransferase.

D. Melissa M. Ruff

(i) Ms. Ruff was a junior research technician in my laboratory in the Howard Hughes Medical Institute at the University of Michigan.

(ii) Under my direct supervision, and under my sole direction, Ms. Ruff completed experiments to optimally transform the *E. coli* host used to construct the cDNA libraries, and used to rescue the vector from the host cells using the Hirt supernatant procedure.

(iii) Under my direct supervision, and under my sole direction, Ms. Ruff completed the actual screening of the libraries. This included each round of transfection, preparation and testing of panning plates, preparation of Hirt supernatants from panned cells, transformation of *E. coli* with the Hirt supernatants, growth of such transformants, and purification of plasmid DNA from such transformants.

(iv) Under my direct supervision, and under my sole direction, Ms. Ruff completed the sibling selection procedure used to isolate the cDNA clones that contained the alpha 1,3-galactosyltransferase cDNA.

(v) Under my direct supervision, and under my sole direction, Ms. Ruff determined the DNA sequence of the alpha 1,3-galactosyltransferase cDNA.

(vi) Ms. Ruff read many of the DNA sequencing gels she had generated.

E. Jolanta Kukowska-Latallo

(i) Dr. Kukowska-Latallo was a post-doctoral fellow in my laboratory in the Howard Hughes Medical Institute at the University of Michigan.

(ii) Under my direction, Dr. Kukowska-Latallo contributed to the transfection of COS cells with alpha 1,3-galactosyltransferase cDNA and the protein A-alpha 1,3-galactosyltransferase expression vectors, and contributed to the FACS analysis of such cells.

F. Richard D. Cummings

(i) Dr. Cummings was an Associate Professor of Biochemistry at the University of Georgia at the time the work described in the Larsen et al. publication was performed.

(ii) Dr. Cummings and I initially had a conversation at the Glycoconjugate Gordon Conference in Santa Barbara, California, in February of 1987. In the course of collaborating with Dr. Cummings, we communicated in writing and by telephone, and we exchanged cell lines and other reagents.

(iii) Dr. Cummings provided the suggestion that the alpha-Gal transferase might be an interesting enzyme to try to clone using the expression cloning strategy that had been developed previously in my laboratory.

(iv) Dr. Cummings provided the suggestion to use the lectin GSIB4 as the "recognition tool" for the specific glycan product of the alpha 1,3-galactosyltransferase, which would be used in the expression strategy that had been previously developed in my laboratory. This strategy had already been successfully implemented in my laboratory, using monoclonal antibodies as the "recognition tool" for other (fucosylated) glycan structures, that were the product of other glycosyltransferases (fucosyltransferases) cloned with this approach.

(v) Dr. Cummings provided the suggestion that retinoic acid differentiated mouse F9 teratocarcinoma cells could be an appropriate source for mRNA for this approach, since Dr. Cummings knew that such cells expressed an alpha 1,3-galactosyl transferase activity.

(vi) Dr. Cummings provided technical and conceptual collaborative contributions in the analysis of the structure of the product of the cloned alpha 1,3-galactosyl transferase. These analyses, including especially the methylation analyses, were completed in Dr. Cummings' laboratory.

(vii) Dr. Cummings provided some editorial comments on the manuscript that ultimately published as the Larsen et al. publication prior to its submission to the journal.

6. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

7. Further deponent saith not.

John B. Lowe

May 1 2003

Date

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